

University of Groningen

Microvascular changes in estrogen-alpha sensitive brainstem structures of aging female hamsters

Gerrits, Peter O.; de Weerd, Henk; van der Want, Johannes J. L.; Kortekaas, Rudie; Luiten, Paul G. M.; Veening, Jan G.

Published in:
Neuroscience Research

DOI:
[10.1016/j.neures.2010.04.010](https://doi.org/10.1016/j.neures.2010.04.010)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2010

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Gerrits, P. O., de Weerd, H., van der Want, J. J. L., Kortekaas, R., Luiten, P. G. M., & Veening, J. G. (2010). Microvascular changes in estrogen-alpha sensitive brainstem structures of aging female hamsters. *Neuroscience Research*, 67(4), 267-274. <https://doi.org/10.1016/j.neures.2010.04.010>

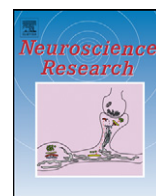
Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.



Microvascular changes in estrogen- α sensitive brainstem structures of aging female hamsters

Peter O. Gerrits^{a,*}, Henk de Weerd^{a,b}, Johannes J.L. van der Want^b, Rudie Kortekaas^c, Paul G.M. Luiten^{d,e}, Jan G. Veening^{a,f,g}

^a Department of Neuroscience, Section Anatomy, University Medical Center Groningen, University of Groningen, A. Deusinglaan 1, 9713 AV Groningen, The Netherlands

^b Department of Cell Biology, Molecular Imaging and Electron Microscopy, University Medical Center Groningen, University of Groningen, A. Deusinglaan 1, 9713 AV Groningen, The Netherlands

^c Department of Neuroscience, Neuroimaging Center, University Medical Center Groningen, University of Groningen, Ant. Deusinglaan 2, 9713 AV Groningen, The Netherlands

^d Department of Molecular Neurobiology, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

^e Department of Biological Psychiatry, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands

^f Department of Anatomy, University Medical Center St Radboud, Nijmegen, Geert Grooteplein 21, 6525 EZ Nijmegen, The Netherlands

^g Department of Psychopharmacology, UIPS, University of Utrecht, Sorbonnelaan 16, 3584 CA Utrecht, The Netherlands

ARTICLE INFO

Article history:

Received 2 February 2010

Received in revised form 15 April 2010

Accepted 16 April 2010

Available online 24 April 2010

Keywords:

Aging

BBB

Endothelial cells

Tight junctions

Basement membrane

Pericyte

(Micro)-vascular degeneration

Gliosis

ABSTRACT

Structural neuronal plasticity is present in the nucleus para-retroambiguus (NPRA) and the commissural nucleus of the solitary tract/A2 group (NTScom/A2) in female hamsters. Both brainstem nuclei play a role in estrous cycle related autonomic adaptations. We investigated how aging affects the capillary condition in these adaptive brainstem regions. Senescent female hamsters (± 95 weeks) were tested weekly for their 4-day estrous cycle. Subsequently morphological changes of NPRA and NTScom/A2 were compared with those of young (± 20 weeks) females in an ultrastructural study. The medial tegmental field served as control area. In 841 capillaries ($n = 319$ capillaries, young females ($N = 3$); $n = 522$ capillaries, aged females ($N = 4$)) vascular aberrations were classified into 3 categories: endothelial and tight junction, basement membrane and pericyte aberrations. In old animals, capillaries showed marked endothelial changes, disrupted tight junctions, and thickening and splitting of basement membranes. Aberrations were found in 40–60% of all capillaries. About 70% of the pericytes contained degenerative inclusions. Despite this generalized vascular degeneration, the reproductive cycle of female hamsters was unaffected by vascular senescence. Perivascular fibrosis as reported in aging rats was never observed, which suggests the existence of species differences.

© 2010 Elsevier Ireland Ltd and the Japan Neuroscience Society. All rights reserved.

1. Introduction

Estrogen receptor- α (ER- α) containing neurons are known to be involved in steroid hormone controlled behaviors including lordosis. There is increasing evidence that estrogen mediates autonomic

and cardiovascular adjustments related to adaptive homeostatic control mechanisms (Saleh et al., 2000, 2005; Saleh and Connell, 2003).

Concerning the vascular effects of estrogen, a large amount of data support the notion that estrogen may play a beneficial role in vascular aging (Miller and Duckles, 2008). More specifically, it has been shown that estradiol treatment has a marked neuroprotective effect in old female gerbils (Wappler et al., 2010). Apparently, estrogens affect endothelial cells and especially their mitochondrial functioning by decreasing the production of reactive oxygen species (Miller and Duckles, 2008). ER- α seems to mediate most of the estrogen effects on endothelial cells (Arnal et al., 2009). Most studies on aging of the vascular and microvascular condition in the mammalian brain focused on cerebral cortical and hippocampal regions, often in relation to neurodegenerative diseases or a compromised cognitive status (Shah and Mooradian, 1997; Farkas and Luiten, 2001). To our knowledge, no specific data are available of

Abbreviations: a, astrocyte; ax, axon; bm, basement membrane; CU, cuneate nucleus; e, endothelium; en, endothelial nucleus; ER- α -IR, estrogen receptor- α -immunoreactive neurons; g, gliosis; IML, intermedialateral cell column; M, mitochondrion; mtf, medial tegmental field; NPRA, nucleus para-retroambiguus; NTScom/A2, commissural part of the solitary tract nucleus/A2 group; p, pericyte process; pd, pericyte degeneration; pn, pericyte nucleus; p, pericyte degeneration; tj, tight junction; Vspcaud, caudal spinal trigeminal complex; WGA-HRP, wheat germ agglutinin-horseradish peroxidase; XP, decussation pyramidal tract; XII, hypoglossal nucleus.

* Corresponding author. Tel.: +31 50 3632488; fax: +31 50 3632461.

E-mail address: p.o.gerrits@med.umcg.nl (P.O. Gerrits).

aging studies on estrogen-receptive in- and output structures in brainstem areas, notwithstanding their importance for the regulation of a myriad of autonomic and motor control functions.

Observations from this laboratory showed that the nucleus para-retroambiguus (NPRA) and commissural nucleus of the solitary tract/catecholaminergic A2 group (NTScom/A2) are part of a brainstem circuit comprising several interrelated nuclei that are subject to functional and structural plasticity and are intimately involved in the regulation of steroid hormone-dependent behaviors and their associated autonomic adaptations (Gerrits et al., 2008a,b, 2009b). In normally aging female hamsters it was found that estrous cycle-induced axonal- and dendritic plasticity of the NPRA and NTScom/A2 remained during aging (Gerrits et al., 2009b). Despite increasing common aging-associated neural and cerebrovascular degenerative changes including blood–brain-barrier impairment, the animals displayed a reproductive behavioral repertoire comparable to a 4-day estrous cycle as observed in young animals (Gerrits et al., 2009a; Veening et al., 2009). Furthermore, in a preliminary exploration, it was noticed that structural aspects of perivascular fibrosis commonly encountered in the hippocampus of aging rats (de Jong et al., 1990a; Farkas et al., 2001) were not seen in the brainstem of the aging hamster (Veening et al., 2009).

Based on these preliminary observations we were particularly interested how aging might affect the capillary structure in specific estrogen receptor rich brainstem centers prone to estrous cycle-dependent neural plasticity. For that reason we analyzed the ultrastructural microvascular changes in the ER- α rich NPRA and NTScom/A2 as compared to the non-ER- α receptive medial tegmental field (mtf, see Fig. 1), in young 'control' females (20 weeks) and aged (95 weeks) female hamsters with an intact reproductive estrous cycle. In addition, to test for possible regional differences, we investigated the occurrence of a specific ultrastructural aberration: the presence or absence of perivascular fibrosis. For that purpose, an anterior region of the brainstem (periaqueductal gray) and the hippocampal formation (CA1 subfield) of the forebrain (Farkas and Luiten, 2001) were screened in both groups of young-control and aging hamsters.

2. Material and methods

2.1. Animals

The experiments started with 10 aging female golden hamsters (*Mesocricetus auratus*), of which eventually four animals met the criteria to be included in the analysis. Three young female hamsters served as controls. In addition, two sexually experienced males were used for the 'estrous-tests' (see Section 2.2). The experiments were performed on inbred animals obtained from Harlan, The Netherlands (strain HsdHan: Aura) weighing 100–120 g. The protocols, surgical procedures, pre- and postoperative care, handling and housing of the animals were in accordance with the ethical guidelines approved by the University Medical Center Groningen, University of Groningen (license number DEC 5142A). All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Experimental procedures

All hamsters were housed individually in plastic cages under a 14/10 h reversed light/dark cycle with food and water available *ad libitum*. Room temperature was maintained at 22–24 °C, and humidity at 50–70%; wood shavings and straw were used as bedding materials. The hamsters were inspected daily and weighed once a week. Females were tested weekly for an invariant 4-day estrous cycle in an observation box in the presence of an exper-

iented adult male. For further details see Gerrits et al. (2008b). Lifespan of hamsters varies considerably from 82 to 118 weeks depending on sex, strain and housing conditions (Kamino et al., 2001; Oklejewicz and Daan, 2002). Therefore, it was decided to euthanize animals at the age of 95–96 weeks, actually at the end of the female hamster lifespan. Vaginal smears were taken to control the period of the cycle (Becker et al., 2005).

2.3. Light and electron microscopy

2.3.1. Perfusion

After an overdose of Nembutal (0.7 ml of 6% sodium pentobarbital, i.p.), the animals were transcardially perfused with 20 ml of heparinized phosphate buffer (0.1 M, pH 7.4), containing 0.4% sodium nitrite and 2% polyvinyl-pyrrolidone (MW 40 K) at 37 °C, followed by 350 ml of fixative containing 0.05% glutaraldehyde, 4% paraformaldehyde, 0.2% picric acid and 2% polyvinyl-pyrrolidone in 0.1 M phosphate buffer, pH 7.4, at room temperature. Following perfusion, the brains were removed and postfixed for 1 h in the same fixative.

2.3.2. Light microscopy

All internal organs were inspected for gross pathology and embedded in Technovit 7100 resin according to Gerrits and Smid (Gerrits and Smid, 1983). 2 μ m thick sections were routinely stained with haematoxylin and eosin, and periodic acid Schiff (PAS).

2.3.3. Electron microscopy

The caudal brainstem tissue was cut on a vibratome into 60 μ m transverse sections and collected in 0.01 M phosphate buffered saline (PBS) at 4 °C. From obex to 2.25 mm caudally, 40 brainstem sections per animal were collected. Every other section was processed for a standard EM protocol: osmicated, dehydrated in a graded series of ethanol and flat-embedded in Epon between dimethyldichlorosilane-coated glass slides. Samples of tissue containing the NPRA, NTScom/A2 and mtf were glued on Epon stubs. After blocking, the tissue was trimmed and cut into 1 μ m semi thin sections. Finally, 60 nm ultrathin sections from the selected areas (NPRA, NTScom/A2 and mtf; Fig. 1) were cut with a diamond knife for further electron microscopical analysis. At the ultrastructural level NPRA and NTScom/A2 neuronal cell bodies, neuropil and surrounding profiles including the vascular system were photographed at 10,000–20,000 \times magnification using a Philips 201 electron microscope (Philips, Eindhoven, The Netherlands), and target structures (blood vessels, perivascular structures and cells) were randomly selected for further analysis. The number of capillaries studied in the old animals was: $n = 522$.

2.3.4. Controls

Brain tissue obtained from young adult female hamsters (20-weeks old; cases H536, H540, H547) served as controls. Electron microscopical processing of tissue was carried out as described above to compare estrous vs. non-estrous dependent brain regions during aging. The number of capillaries studied in the young-control animals was: $n = 319$.

2.4. Quantitative analysis

Photomicrographs were collected at random from thin sections of NPRA, NTScom/A2 and mtf, respectively, at four different depth intervals of 1 μ m. 841 different profiles of capillaries (young females ($N = 3$), $n = 319$; aged females ($N = 4$), $n = 522$) were screened with emphasis on various forms of vascular degeneration. Microvascular aberrations were classified into three categories: (1) endothelial inclusions, edematous endothelium, and tight junction

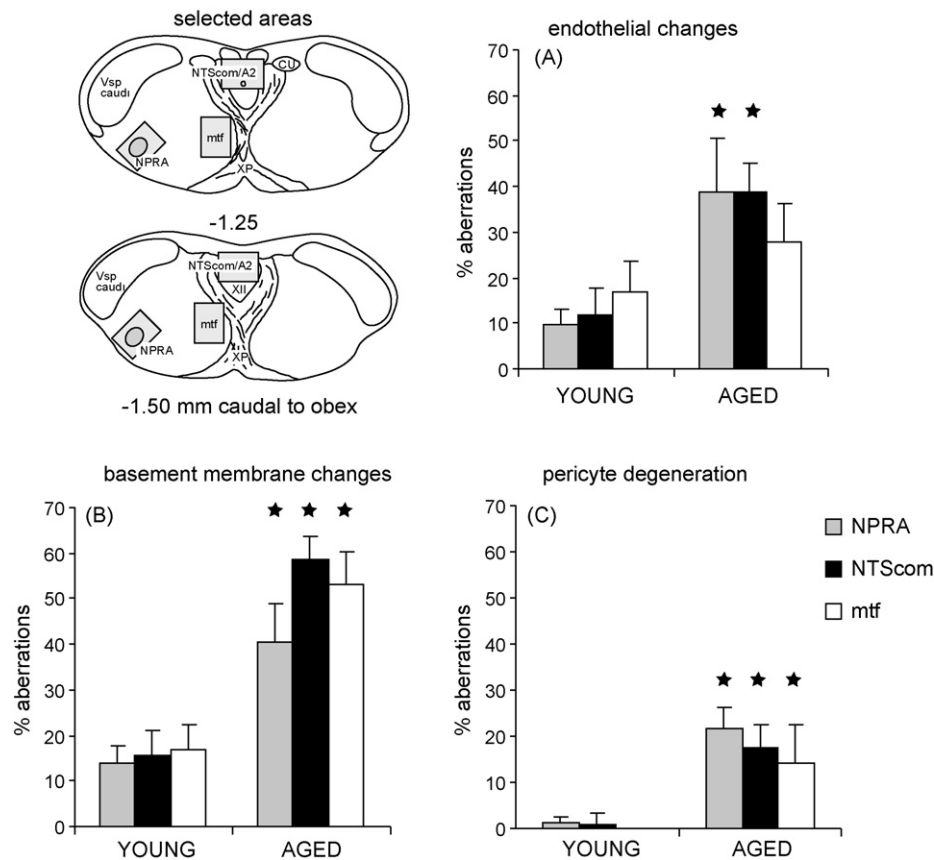


Fig. 1. Schematic representation of the three different brainstem locations selected for morphometrical analysis, nucleus para-retroambiguus (NPRA), commissural nucleus of the solitary tract/A2 group (NTScom/A2), and medial tegmental field (mtf). Histograms A–C shows the percentage of capillaries with endothelial changes (A), basement membrane changes (B) and pericyte aberrations (C), in young and aged hamsters, respectively. * $p < 0.01$ – 0.04 (see text).

impairment (END); (2) basement membrane aberrations (thickening/splitting, BM) and (3) pericyte degeneration (PD). Inside the END-category, a total of 637 tight junctions were screened for aberrations in young ($n = 296$) and in aged females ($n = 341$).

2.4.1. Statistical analysis

Statistical analysis consisted of a repeated measures ANOVA with three dependent variables: PD, END and BM. Age was defined as a categorical between-subjects factor of two levels and location as a categorical within-subjects factor of three levels. Separate observations from within one animal were treated as dependent in order to maintain statistical stringency by preventing inflation of degrees of freedom.

3. Results

Experimental females ($N = 4$) maintained a normal estrous cycle up till 95–96 weeks of age. Vaginal smears gave further evidence for intact cyclicity. The animals weighed on average 135 g and displayed a normal healthy condition. Gross anatomical examination of the viscera showed a small liver cyst in one female and two small bilateral ovary cysts in another animal.

3.1. Changes in capillary ultrastructure

NPRA and NTScom/A2 and medial tegmental field showed diffuse neurodegenerative changes varying from increased amounts of neuronal intracytoplasmic lipofuscin, various forms of abnormal mitochondria, degenerated myelin accumulations, age-related bodies and gliosis. Fig. 1A–C illustrates the distribution of microvas-

cular aberrations in young and aged animals. Capillaries with marked endothelial changes often in combination with disrupted tight junctions, thickened basement membranes, pericyte degeneration and perivascular gliosis and astrocytic endfeet swelling were observed. Isolated or combined these aberrations were present in 40–60% of all capillaries in NPRA and NTScom/A2. Multivariate statistical analysis showed that age had a strong effect on endothelial changes and basement membrane changes as well as pericyte degeneration [$F(3,3) = 35.251$, $p = 0.008$].

3.1.1. Endothelial cells and tight junctions

Endothelial changes were observed in about 40% of the capillaries in the area of NPRA and NTScom/A2. However, in the mtf these changes were less frequently observed in about 30% of the capillaries (Fig. 1A). Fig. 2A–D shows distinct stages of endothelial degeneration and disruption of tight junctional complexes. Damaged endothelium shows elements of edematous cytoplasm and extra-cellular swelling with small vesicles and fine tubular structures. Disrupted tight junctions were found in young adult control cases as well as in aged hamsters (Fig. 2A and B). Three times more aberrations were found in the latter (21.1% vs. 6.8% of all screened tight junctions). Age had a significant effect on endothelial impairment [$F(1,5) = 40.59$, $p = 0.001$]. There was no overall location effect in the multivariate test [$F(6,18) = 2.032$, $p = 0.114$] and no location effect on endothelial impairment when comparing NPRA/NTScom/A2 vs. mtf.

3.1.2. Basement membrane impairment

Generalized elements of basement membrane aberrations (BM) were observed (Fig. 3A–D). In Fig. 3A the capillary

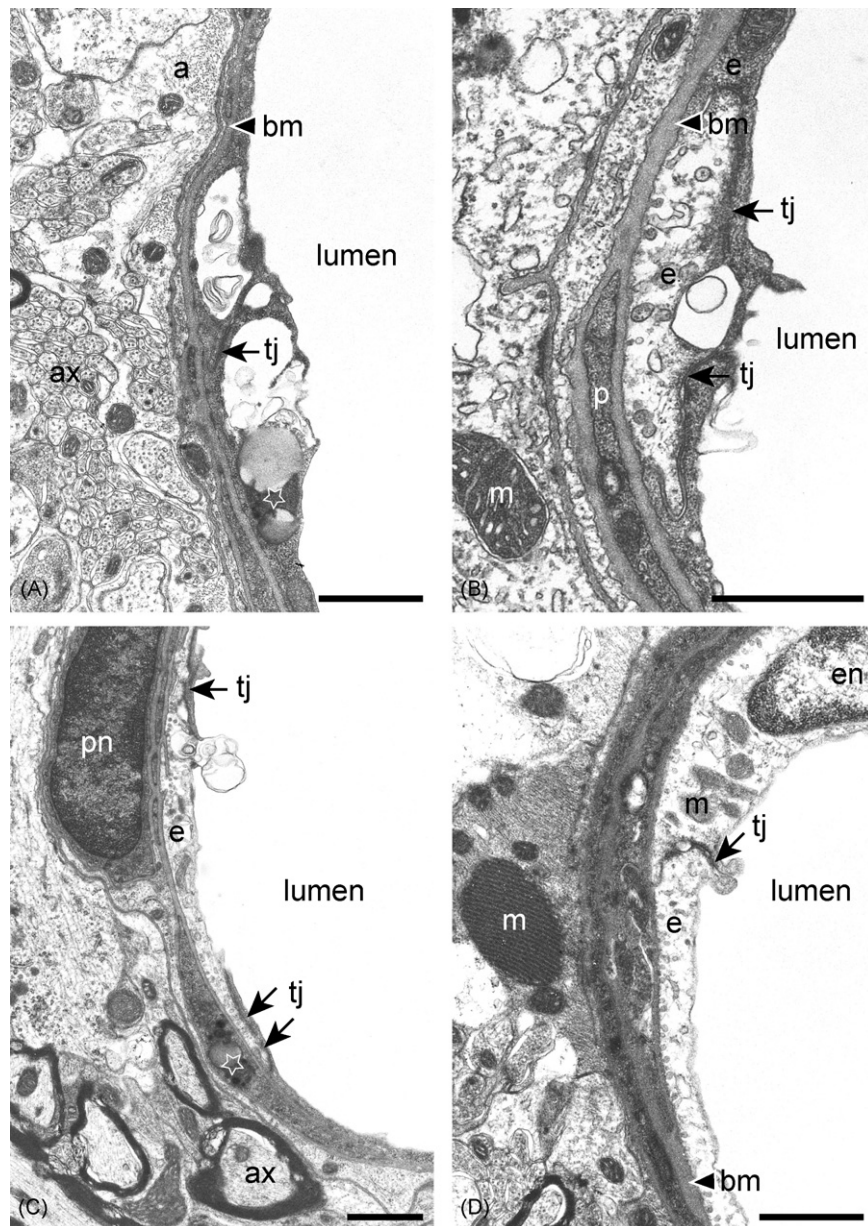


Fig. 2. (A–D) Electron microscopic photomicrographs showing stages of endothelial degeneration and structural changes of tight junctional complexes in female aged hamsters. (A) Shows an electron photomicrograph of part of a capillary (lumen) in NTScom/A2 surrounded by a thin, endothelial cell layer that forms a tight junction (tj) complex between two opposing membrane protrusions. The endothelial layer shows electron lucent vacuoles that contain membrane deposits and amorphous accumulations (white asterisk). The basement membrane (bm) is composed of several layers running in parallel. Numerous unmyelinated axons (ax), and astrocyte end feet (a) are present. Mitochondria (m) show a relatively dark matrix with pale cristae. Case H571. (B) Endothelial (e) lining covers the lumen of a capillary in the NPRA. The edematous cytoplasm of the endothelial cell shows small vesicles and fine tubular elements. Tight junctions (tj) can be observed between overlapping cellular extensions. A pericyte process (p) is located between two layers of irregular thickened basement membrane (bm). A mitochondrion (m) in an adjacent astrocyte shows a dark matrix and irregular cristae (H574). (C) mtf. In the lumen of a capillary tight junctions (tj) are formed between overlapping endothelial (e) membrane extensions (arrows). Endothelial content at the level of tj is bulging into the lumen. Degenerative inclusions (white asterisk) are present in the cytoplasm of a pericyte. A pericyte with large nucleus (pn) is located next to the basement membrane. In the neuropil myelinated axons (ax) can be observed. (D) NPRA. Increased thickening of the basement membrane (bm) next to the endothelial cell (e) contains a prominent nucleus (en) and some small mitochondria (black m). The cell adjacent to the endothelial cell shows a mitochondrion (white m) with a crystalline-like pattern of cristae, whereas others show a more random organization of the cristae. Case H571. Bars in A–D: 1 μ m.

basement membrane is regular but displays a gradual thickening (arrow heads) with some thin membrane extensions pointing into the surrounding tissue. Furthermore, irregular basement membrane thickening with 'sprouting' protrusions extending into the deeper cell layers of the neuropil were found (Fig. 3B). Quantitative analysis showed that age had a marked effect on BM [$F(1,5)=135.0$, $p=0.000$] (Fig. 1B). There was no overall location effect in the multivariate test [$F(6,18)=2.032$, $p=0.114$]. However, when data were analyzed for basement membrane aberrations separately, there was a loca-

tion effect: basement membrane changes were less pronounced in NPRA.

3.1.3. Pericyte degeneration

In old animals about 70% of all pericytes observed contained degenerative inclusions (PD), in contrast to young-control animals, where almost no aberrant pericytes were observed. The analysis per capillary included all capillaries even where no surrounding pericyte could be discerned (Fig. 1C). This figure shows that more than 20% of the capillaries showed degenerating pericytes, while in

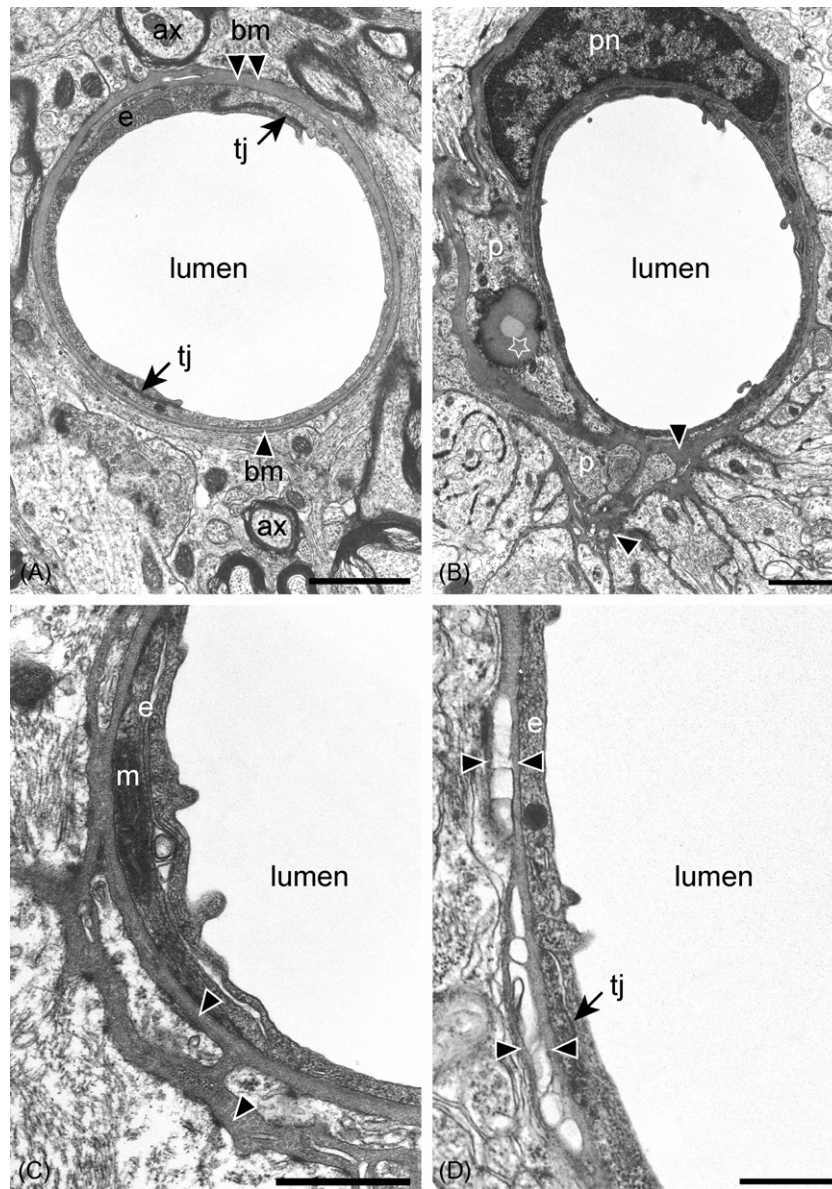


Fig. 3. (A–D) Different aspects of basement membrane (bm) impairment, irregular membrane thickness and endothelial cell (e) structure. (A) Capillary basement membrane in NPRA (H576) showing a pale appearance with a marked circular outline, arrowheads indicate differences in thickness. (B) NTScom/A2, the basement membrane in this case (H574) is much denser and irregular with protrusions that reach in between deeper cell layers in the neuropil. Pericytes (p) are covered with basement membrane extensions protruding between axonal and astrocytic processes. In the cytoplasm of pericytes large vacuoles are present that contain dense, autophagic inclusions (white asterisk). (C) Area NTScom/A2, (H574) shows a higher magnification of an irregularly thickened basement membrane (arrow heads) that is apposed to the endothelium (e). Note the presence of perivascular gliosis. In (D) the capillary basement membrane is thickened and splitted up and contains membranous inclusions (between arrow heads). NTScom/A2, case H574. Abbreviations: m, mitochondrion, tj, tight junction, p, pericyte. Bars in A–D: 1 μ m.

young animals such phenomena are virtually absent. In degenerative pericytes the cytoplasm was frequently packed densely with lysosome-like structures containing electron dense deposits with different structural densities (Fig. 4A and B). At the light microscopical level these structures were PAS-positive and as such indicative for lipofuscin. Quantitative analysis revealed that increasing age had a significant effect on pericyte degeneration [$F(1,5)=29.41$, $p=0.003$]. There was no overall location effect in the multivariate test [$F(6,18)=2.032$, $p=0.114$] and no location effect on pericyte degeneration.

3.1.4. Perivascular aberrations

Degenerative changes in the capillary wall as described above were often associated with other aberrations in close proximity of the vessel such as edematous astrocytic endfeet swelling (Fig. 4C),

gliosis and proliferation of lamellar structures (Fig. 4D). No areas with generalized perivascular edema were found.

3.1.4.1. Comparative regional observations: caudal brainstem vs. PAG and hippocampus. To exclude possible occurrence of regional differences in vascular aging patterns, especially the occurrence of perivascular fibrosis phenomena, two additional brain areas were screened as well: PAG and hippocampal CA1 subfield. 120 capillaries were screened in both areas. Aged-induced capillary changes in these brain structures turned out to be very similar to those described in the caudal brainstem. Amorphous perivascular fibrosis, or fibrosis with discernible fiber formations, as described in aging rats were never observed in the NPRA, NTScom/A2, medial tegmental field, or the PAG and hippocampal CA1 region.

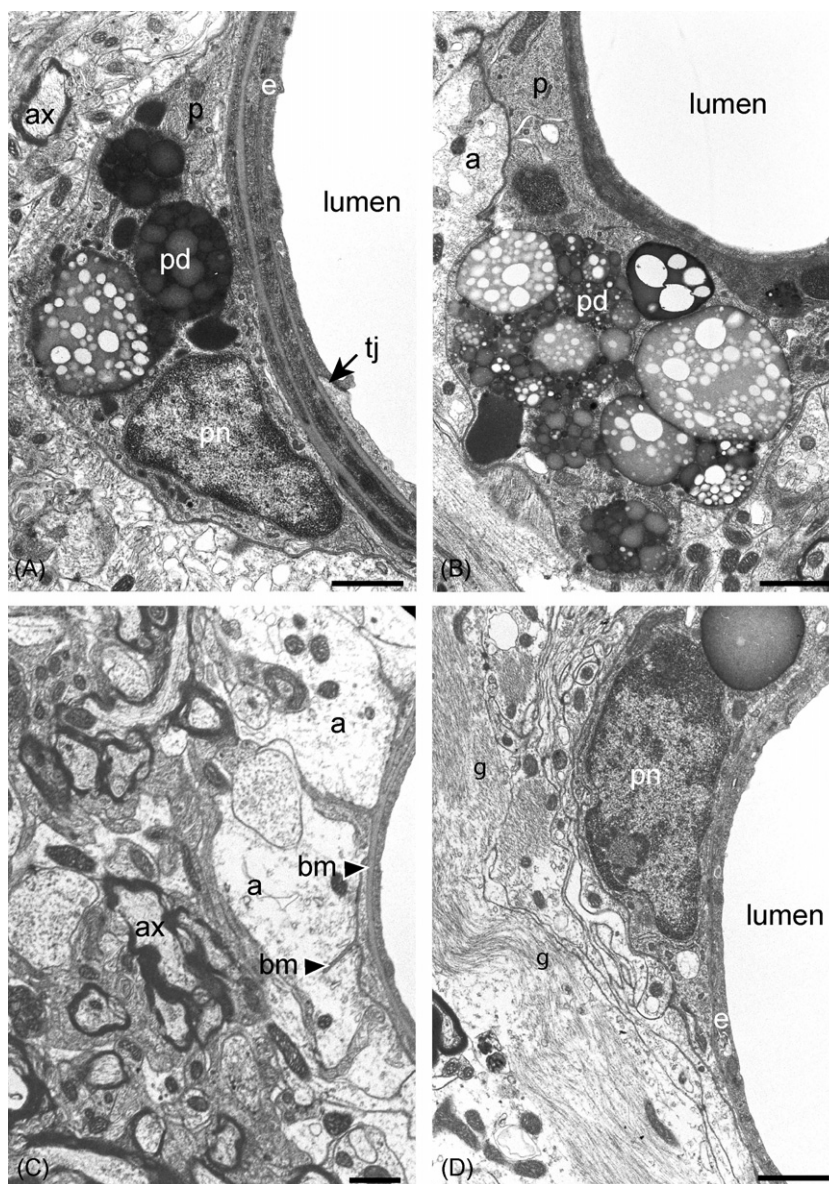


Fig. 4. (A–D) Degenerative changes in pericytes and astrocytes in the vicinity of blood vessels (lumen). Examples of degenerative changes in pericytes are shown in A and B (cases H571 and 574). The cytoplasm of the pericytes (p) is densely filled with lysosome-like bodies that contain electron dense deposits (pd) with variable structural densities. (C) mtf: basement membrane with remarkable extensions (bm, arrow head) indwelling into the cytoplasm of swollen astrocytes (a), H574. In (D) extensive gliosis (g) is present in the neuropil of NPRA (H571), and a large vacuolar inclusion can be observed in a pericyte (pericyte nucleus: pn). Bars in A–D: 1 μ m.

3.1.5. Control observations: young vs. old animals

Brain tissue of the young animals ($N=3$) was processed exactly similar to the aged animals and served to control for possible aberrations as a result of the perfusion fixation, and as a comparison group for the aging-associated microvascular changes. No perfusion-related damage could be observed and microvascular changes were significantly less frequent than in old animals (Fig. 1A–C). In the young animals only about 10–15% aberrations were found in endothelium and basement membranes, while pericyte degeneration or perivascular gliosis was hardly observed at all.

To summarize the results of the present experiments: there is a remarkable similarity in the preservation of ultrastructural vascular details. No differences were observed between the microvascular changes occurring in the estrogen-receptive vs. the estrogen-non-receptive caudal brain stem areas of the female hamster brain. The perivascular fibrosis phenomena, as observed in the forebrain of the rat, were never observed in the hamster brain,

neither in the caudal brainstem nor in the PAG and hippocampal areas.

4. Discussion

In the present study, we compared the microvascular fine structure during aging in estrogen-receptive brainstem areas with those of non-receptive reproductive regions in the female hamster brain. While the female hamsters maintained reproductive cycling during senescence, no differences were observed in either the frequency or the characteristics of the microvascular aberrations between receptive and non-receptive brain areas. Apparently, estrogen-sensitivity of a particular brain region is unrelated and provides no specific protection for its vascular components against age-induced degenerative changes. In addition, no regional differences were observed in microvascular aberrations between brainstem and forebrain levels of the aging female hamster brain. This finding suggests possible species differences in microvascular aging effects,

as in rats specific forms of perivascular fibrosis have been reported which were never detected in the aging hamster brain (Farkas and Luiten, 2001).

4.1. Estrogen-receptive brainstem areas in the female hamster brain

We investigated how aging affects microvascular profiles in the estrogen-receptive NPRA and NTScom/A2 and compared these brainstem areas with forebrain regions reportedly sensitive to brain aging. At ultrastructural level no significant differences were found. The neural tissue of all aged animals showed similar extensive degenerative aberrations in all investigated brain areas. These aberrations ranged from increased cytoplasmic lipofuscin, abnormal and giant mitochondria, various forms of myelin degeneration and considerable numbers of age-related bodies, to perivascular gliosis and indications for blood–brain-barrier (BBB) degeneration (Gerrits et al., 2009a,b; Veening et al., 2009). A comparison of our findings with previously reported data of other mammalian species turns out to be difficult, since studies of senescent hamsters are scarce (Navarro et al., 1996).

4.2. Blood–brain-barrier impairment and microvascular aberrations

In rat, monkey and human perivascular changes include various forms of fibrosis (de Jong et al., 1990a; Farkas and Luiten, 2001; Knox et al., 1980), gliosis (Ravens, 1978), membranous debris within the basement membrane (Casey and Feldman, 1985) and basement membrane thickening (Casey and Feldman, 1985; de Jong et al., 1990a; Knox and Oliveira, 1980; Farkas et al., 2001).

Deranged parts of the BBB may directly or indirectly lead to impairment of neuronal functioning. In addition, some characteristics of BBB-aberrations appear to be even region specific (Goldman et al., 1992; Nandy et al., 1975; Shah and Mooradian, 1997; Threatt et al., 1971; Zlokovic, 2008). In agreement with Wadhwani et al. (1991) we found no areas with generalized perivascular edema in senescent hamsters. On the other hand, edematous astrocytic endfeet swelling observed in close proximity of capillaries (Fig. 4C) is suggestive for locally enhanced BBB permeability.

4.2.1. Endothelium and tight junction

Aging had a significant effect on endothelial integrity. Impaired endothelium displays edematous cytoplasm, small vesicles and/or fine tubular structures. Loss of cytoplasmic content could be related to loosening of tight junctional integrity, or endothelial plasma membrane disruption, leading to increased paracellular permeability. Tight junction aberrations were found in young adult control cases as well as in senescent hamsters. In the aged group the amount of abnormal tight junctions was three times the number in young adults but region specific changes were not observed. It can be concluded that endothelial and tight junction changes in the estrogen-receptive areas involved in reproduction were not different from the other brain areas investigated.

4.2.2. Basement membrane

In rat, aging was shown to be associated with increased basal laminar thickening of hippocampal capillaries (Topple et al., 1991), which changes were partially reversed after chronic treatment with the calcium channel antagonist nimodipine, indicating a calcium dependent aging mechanism (de Jong et al., 1990b).

In the aging hamster various common elements of basement membrane aberrations were observed. First, capillaries were found in which the basement membrane is regular but displays a gradually thickening around the vessel with thin membrane extensions

invading into the surrounding neuronal tissue. Further, many irregular basement membrane thickenings were observed, similar to those described in the rat (de Jong et al., 1990a; Farkas et al., 2001; Farkas and Luiten, 2001). Finally, irregular basement membrane thickenings were present in combination with protrusions extending into the deeper cell layers of the neuropil (Fig. 3B). Our analysis revealed no differences between estrogen-rich reproduction brainstem areas and 'control' brain(stem) regions, suggesting that estrogen-sensitivity of a given brainstem area provides no 'protection' against microvascular aging-associated changes.

4.2.3. Pericytes

Pericytes are the nearest neighbours of endothelial cells, with which they share a common basement membrane. They intimately embrace the abluminal surface of endothelial cells and cover 22–32% of cerebral capillary surface (Allt and Lawrenson, 2001). In addition to their supposed phagocytotic actions, pericytes demonstrate high contents of muscle and non-muscle actins suggesting a contractile function with blood flow regulating abilities (Allt and Lawrenson, 2001; Herman and D'Amore, 1985). This was confirmed by Peppiatt et al. (2006) in a study on bidirectional control of CNS capillary diameter by pericytes.

In our old animals 70% of all pericytes contained densely packed degenerative inclusions with a lysosome-like appearance and with a different electron density. Quantitative analysis revealed that age had a significant effect on pericyte degeneration in each of the brainstem areas investigated. Since pericytes are probably modulators of blood flow in response to changes in neural activity, it is very likely that massive pericyte degeneration has serious consequences for local blood flow regulation. No differences in pericyte degeneration were observed in estrogen-receptive and the other 'control' brain(stem) areas suggesting that this factor does not play a significant role in these age-associated changes.

4.2.4. Astrocytes and other perivascular structures

The abluminal vascular surface of brain capillaries is densely covered by perivascular astrocytic endfeet. We observed that damage of the capillary wall and/or the presence degenerative pericytes was often associated with other aberrations in close proximity of the vessel wall, including astrocytic endfeet swelling, massive gliosis and proliferation of lamellar structures as most striking features.

4.3. Specificity of the aberrations: region or species related?

The capillary changes as described above were observed to the same extent in each of the screened areas in posterior and anterior brainstem and hippocampus, so there was no evidence for region specific differences in microvascular degeneration during the aging process in the female hamster. However, some characteristic forms of perivascular fibrosis present as amorphous or structured collagen depositions as described for the rat and human brain (de Jong et al., 1990a; Farkas et al., 2001; Farkas and Luiten, 2001) were not found in any of these areas of the aged hamster brain. This striking difference suggests that species specific vascular aging processes occur, which warrants further investigation of the mechanisms, involved in microvascular degeneration processes.

Acknowledgements

We would like to thank Angelika Jurdzinski for animal care and welfare, Anneke Algra for help with morphometrical analysis of this work, and Dirk Huizinga, for helping with electron photomicrographs. Part of these data were presented in preliminary form at the Society for Neuroscience 2009 832.28 (Chicago).

References

- Allt, G., Lawrenson, J.G., 2001. Pericytes: cell biology and pathology. *Cells Tissues Organs* 169, 1–11.
- Arnal, J.F., Laurell, H., Fontaine, C., Billon, A., Calippe, B., Lenfant, F., Gourdy, P., 2009. Estrogen receptor actions on vascular biology and inflammation: implications in vascular pathophysiology. *Climacteric* 12 (Suppl. 1), 12–17.
- Becker, J.B., Arnold, A.P., Berkley, K.J., Blaustein, J.D., Eckel, L.A., Hampson, E., Herman, J.P., Marts, S., Sadée, W., Steiner, M., Taylor, J., Young, E., 2005. Strategies and methods for research on sex differences in brain and behavior. *Endocrinology* 146, 1650–1673.
- Casey, M.A., Feldman, M.L., 1985. Aging in the rat medial nucleus of the trapezoid body. II. Electron microscopy. *J. Comp. Neurol.* 232, 401–413.
- de Jong, G.I., de Weerd, H., Schuurman, T., Traber, J., Luiten, P.G., 1990a. Microvascular changes in aged rat forebrain. Effects of chronic nimodipine treatment. *Neurobiol. Aging* 11, 381–389.
- de Jong, G.I., Horvath, E., Luiten, P.G., 1990b. Effects of early onset of nimodipine treatment on microvascular integrity in the aging rat brain. *Stroke* 21, IV113–IV116.
- Farkas, E., De Jong, G.I., Apro, E., Keuer, J.I., Luiten, P.G., 2001. Calcium antagonists decrease capillary wall damage in aging hypertensive rat brain. *Neurobiol. Aging* 22, 299–309.
- Farkas, E., Luiten, P.G., 2001. Cerebral microvascular pathology in aging and Alzheimer's disease. *Prog. Neurobiol.* 64, 575–611.
- Gerrits, P.O., Kortekaas, R., Algra, A., van der Want, J.J.G., Veening, J.G., 2009a. Neurodegeneration and Plasticity in the Brainstem of Estrous Female Golden Hamster; Ultrastructural Studies on Axo-dendritic Relationships, Remodeling and Degeneration. Society for Neuroscience, Chicago.
- Gerrits, P.O., Kortekaas, R., Veening, J.G., de Weerd, H., Algra, A., Mouton, L.J., van der Want, J.J., 2008a. Estrous cycle-dependent neural plasticity in the caudal brainstem in the female golden hamster: ultrastructural and immunocytochemical studies of axo-dendritic relationships and dynamic remodeling. *Horm. Behav.* 54, 627–639.
- Gerrits, P.O., Krukerink, M., Veening, J.G., 2009b. Columnar organization of estrogen receptor-alpha immunoreactive neurons in the periaqueductal gray projecting to the nucleus para-retroambiguus in the caudal brainstem of the female golden hamster. *Neuroscience* 161, 459–474.
- Gerrits, P.O., Smid, L., 1983. A new, less toxic polymerization system for the embedding of soft tissues in glycol methacrylate and subsequent preparing of serial sections. *J. Microsc.* 132, 81–85.
- Gerrits, P.O., Veening, J.G., Blomsma, S.A., Mouton, L.J., 2008b. The nucleus para-retroambiguus: a new group of estrogen receptive cells in the caudal ventrolateral medulla of the female golden hamster. *Horm. Behav.* 53, 329–341.
- Goldman, H., Berman, R.F., Gershon, S., Murphy, S., Morehead, M., Altman, H.J., 1992. Cerebrovascular permeability and cognition in the aging rat. *Neurobiol. Aging* 13, 57–62.
- Herman, I.M., D'Amore, P.A., 1985. Microvascular pericytes contain muscle and non-muscle actins. *J. Cell Biol.* 101, 43–52.
- Kamino, K., Tillmann, T., Mohr, U., 2001. Spectrum and age-related incidence of spontaneous tumours in a colony of Han:AURA hamsters. *Exp. Toxicol. Pathol.* 52, 539–544.
- Knox, C.A., Yates, R.D., Chen, I., Klara, P.M., 1980. Effects of aging on the structural and permeability characteristics of cerebrovasculature in normotensive and hypertensive strains of rats. *Acta Neuropathol.* 51, 1–13.
- Miller, V.M., Duckles, S.P., 2008. Vascular actions of estrogens: functional implications. *Pharmacol. Rev.* 60 (2), 210–241 (Epub 2008 Jun 25).
- Nandy, K., Fritz, R.B., Threath, J., 1975. Specificity of brain-reactive antibodies in serum of old mice. *J. Gerontol.* 30, 269–274.
- Navarro, A., Gonzalez del Rey, C., Tolivia, J., Alvarez-Uria, M., 1996. Ultrastructural and quantitative study of atypical age-related bodies in the hamster brain. *Mech. Ageing Dev.* 90, 75–90.
- Oklejewicz, M., Daan, S., 2002. Enhanced longevity in tau mutant Syrian hamsters, *Mesocricetus auratus*. *J. Biol. Rhythms* 17, 210–216.
- Peppiatt, C.M., Howarth, C., Mobbs, P., Attwell, D., 2006. Bidirectional control of CNS capillary diameter by pericytes. *Nature* 443, 700–704.
- Ravens, J.R., 1978. Vascular changes in the human senile brain. *Adv. Neurol.* 20, 487–501.
- Saleh, M.C., Connell, B.J., Saleh, T.M., 2000. Autonomic and cardiovascular reflex responses to central estrogen injection in ovariectomized female rats. *Brain Res.* 879, 105–114.
- Saleh, T.M., Connell, B.J., 2003. Central nuclei mediating estrogen-induced changes in autonomic tone and baroreceptor reflex in male rats. *Brain Res.* 961, 190–200.
- Saleh, T.M., Connell, B.J., Cribb, A.E., 2005. Sympathoexcitatory effects of estrogen in the insular cortex are mediated by GABA. *Brain Res.* 1037, 114–122.
- Shah, G.N., Mooradian, A.D., 1997. Age-related changes in the blood-brain barrier. *Exp. Gerontol.* 32, 501–519.
- Threath, J., Nandy, K., Fritz, R., 1971. Brain-reactive antibodies in serum of old mice demonstrated by immunofluorescence. *J. Gerontol.* 26, 316–323.
- Topple, A., Fikova, E., Baumgardner, D., Cullen-Dockstader, K., 1991. Effect of age on blood vessels and neurovascular appositions in the CA1 region of the rat hippocampus. *Neurobiol. Aging* 12, 211–217.
- Veening, J.G., De Weerd, H., Van der Want, J.J.L., Luiten, P.G.M., Gerrits, P.O., 2009. Microvascular Changes in the Brainstem of Aging Female Hamsters. Society for Neuroscience, Chicago.
- Wadhvani, K.C., Koistinaho, J., Balbo, A., Rapoport, 1991. S.I. Blood-nerve and blood-brain barrier permeabilities and nerve vascular space in Fischer-344 rats of different ages. *Mech. Ageing Dev.* 58, 177–190.
- Wappler, E.A., Felszeghy, K., Szilágyi, G., Gál, A., Skopál, J., Mehra, R.D., Nyakas, C., Nagy, Z., 2010. Neuroprotective effects of estrogen treatment on ischemia-induced behavioural deficits in ovariectomized gerbils at different ages. *Behav. Brain Res.* 1 209 (1), 42–88 (Epub 2010 Jan 18).